Crop Rotation and Nitrogen Input Effects on Soil Fertility, Maize Mineral Nutrition, Yield, and Seed Composition

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ABSTRACT

Knowledge of complex relationships between soils, crops, and management practices is necessary to develop sustainable agricultural production systems. Objectives were to determine how maize (*Zea mays* L.) would respond to monoculture (C-C), 2-yr rotation (C-S) with soybean [*Glycine max* (L.) Merr.], or 4-yr rotation (C-S-W/A-A) with soybean, wheat (*Triticum aestivum* L.), and alfalfa (*Medicago sativa* L.) under different N input levels. We evaluated N fertilizer input (8.5 or 5.3 Mg/ha yield goal, or no N) and crop rotation (C-C, C-S, or C-S-W/A-A) treatment effects on soil minerals (N, P, K, S, Ca, Mg, Fe, Mn, and Zn) and their subsequent effect on shoot dry weight and mineral concentrations, grain yield, and grain composition (oil, starch, and mineral concentrations) using univariate and multivariate statistical tests. Soil under C-S-W/A-A rotation had greater NO_3–N and less extractable P than other rotations. Significant input × rotation interactions revealed that shoot concentrations of N, Ca, and Mg were less while P, K, and Zn were greater at no N input for the C-C rotation compared with other N input/rotation treatments. Increased soil NO_3–N, increased plant Ca concentration, and increased grain N and grain S concentrations were most important in differentiating C-S-W/A-A rotation from C-C and C-S rotation treatments. No N input resulted in less yield and kernel N concentration within the C-C and C-S rotations but not C-S-W/A-A. Thus, growing maize in extended rotations that include forage legumes may be a more sustainable practice than growing maize in either monoculture or 2-yr rotation with soybean.

Soil N levels often increase when N-fixing legumes are included as rotation crops (Peterson and Varvel, 1989a; Raimbault and Vyn, 1991; Carpenter-Boggs et al., 2000). Deep-rooted legume crops, such as alfalfa, scavenge deep residual soil N and thus increase N availability to subsequent shallow-rooted crops (Mathers et al., 1975; Karlen et al., 1994). Although there are obvious effects of rotation on soil mineral status, particularly N, researchers have concluded that there is a rotation effect beyond that which can be explained by soil mineral status alone (Wright, 1990; Bullock, 1992; Copeland and Crookston, 1992). Crop rotation improved soil structure (Raimbault and Vyn, 1991), increased soil organic matter levels (Campbell and Zentner, 1993; Bremer et al., 2008), increased water use efficiency (Roder et al., 1989; Varvel, 1994; Tanaka et al., 2005), enhanced mycorrhizal associations (Johnson et al., 1992), improved grain quality (Kaye et al., 2007), and reduced grain yield variability (Varvel, 2000). Crop rotations also provide better weed control, interrupt insect and disease cycles, and improve crop nutrient use efficiency (Karlen et al., 1994).

When grown in rotation, maize grain yield was 10 to 17% greater than monoculture (Mannering and Griffith, 1981; Dick et al., 1986; Higgs et al., 1990). Significant increases in yield for maize grown in rotation were also recorded in experiments where N, P, and K soil test levels were high and pest populations were managed (Bolton et al., 1976; Copeland and Crookston, 1992; Higgs et al., 1976; Welch, 1976). Thus the rotation effect can have substantial positive influence on maize yields (Peterson and Varvel, 1989b). Root function was improved (Copeland et al., 1993) and plant uptake of N, P, K, and Ca was increased (Copeland and Crookston, 1992; Riedell et al., 1998) in maize grown in rotation with soybean when compared with monoculture maize.

We were interested to see if these same effects would take place under more complex rotations that included wheat and alfalfa. Understanding the complex interactions of soils, plants, and management practices is a first step toward development of agricultural systems that conserve soil and water resources while sustaining crop production. Thus, we were also interested in identifying soil, plant, and grain mineral nutrient variables that best discriminate N input treatments as well as rotation treatments from one another. The objectives of this study were to determine how soil mineral nutrients as well as maize growth, shoot and grain mineral nutrients, and yield would respond to monoculture, 2-yr C-S rotation, or 4-yr C-S-W/A-A rotation under different N input levels using univariate and multivariate statistical techniques.

MATERIALS AND METHODS

Study Site and Experiment Treatments

This field study was conducted at the Eastern South Dakota Soil and Water Research Farm near Brookings, SD (44° 19′ N,

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Published in Agron. J. 101:870–879 (2009).
doi:10.2134/agronj2008.0186x.

Abbreviations: ICP–AES, inductively coupled plasma–atomic emission spectrometer; NP, nitrogen prescription; VT, tassel development stage; YG, yield goals.

96° 46′ W; 500 m elevation) during the 1998 and 1999 growing seasons. The Barnes loam (fine-loamy, mixed Udic Haploborolls with nearly-level topography; Maursetter et al., 1992) soils on the farm are characteristic of those found in eastern South Dakota and western Minnesota and are similar to soils common to the northern U.S. maize belt. Crop rotation and N application rate treatments began in 1990. Primary tillage was by moldboard plow in the fall, weather permitting, or in spring otherwise. After spring 1996, a chisel plow operation replaced the moldboard plow. Triple super phosphate as 0–20–0 (elemental N-P-K) equivalent to 89 kg ha^{-1} of elemental P was broadcast on all plots before spring field work in 1996 (Pikul et al., 2005). Plots were prepared each spring using a tandem disk and field cultivator. All maize and soybean plots were row-cultivated twice each year to a soil depth of about 8 cm using a JD885 row crop cultivator (Deere & Company, Moline IL).

The experiment consisted of three replicate blocks of three crop rotations: continuous maize monoculture (C-C), a 2-yr maize-soybean rotation (C-S), and a 4-yr maize-soybean-wheat/alfalfa-alfalfa rotation (C-S-W/A-A). The whole-plot experimental units, rotation treatments, were arranged in a randomized complete block design. Subplot treatments (N input level) were randomly assigned to each whole plot. In the C-S-W/A-A rotation, spring wheat was a grain crop as well as a companion crop to establish alfalfa in Year 3 of the rotation. Alfalfa was cut for hay in the fourth year. All crops in rotation were present each year. Each 90 by 30 m rotation plot was split into three randomized subplots (30 by 30 m) to test N fertilization effects at no N, intermediate N, and high N application rates. Fertilizer N application rates for the maize phase of the rotation were based on yield goals (YG) of 0 (no N), 5.3 (intermediate N), and 8.5 (high N) Mg ha^{-1}. A preseason (17 Oct. 1997; 20 Oct. 1998) total soil nitrate-nitrogen test (TSN) was used to estimate fertilizer N prescription for the maize phase of the rotation (Gerwing and Gelderman, 1996). For each N treatment level, nitrogen prescription (NP) was calculated as NP = 0.022YG– TSN (Pikul et al., 2005). No adjustments were made to the NP for previous crop (N credits) or sampling date.

Starter fertilizer was applied to all plots in maize, soybean, and wheat rotation phases. Starter fertilizer contained 18 kg ha^{-1} P, 12 kg ha^{-1} K, and 0, 8, or 16 kg ha^{-1} N in the no N, intermediate N, or high N plots, respectively. Starter fertilizer was banded 5 cm to the side and 5 cm deep from the seed furrow for maize and soybean crops. For wheat, starter was applied in the seed furrow. After subtracting preplant soil NO_3–N and starter fertilizer from the NP, the remaining N requirement in maize was applied (sidedress operation) as urea (46–0-0) just before the second row-cultivation operation (16 June 1998, DOY 167; 21 June 1999, DOY 172). The alfalfa phase of the C-S-W/A-A rotation received no fertilizer. Additional information on field plot and crop management procedures used in this study can be obtained from Riedell et al. (1998) and Pikul et al. (2001; 2005).

Maize (Pioneer 3751) was planted using an eight-row planter with 76-cm row spacing on 30 Apr. 1998 (DOY 120) and 12 May 1999 (DOY 132). Average seeding rate was 87,000 seeds ha^{-1}. Alachlor (3.7 L ha^{-1}) [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide] and bromoxynil (0.7 L ha^{-1}) (3,5-dibromo-4-hydroxybenzonitrile) were applied for weed control. No insecticides were applied. All plots were harvested on 8 Oct. 1998 (DOY 281) or 6 Oct. 1999 (DOY 279).

Soil Measurements

Soil samples to a depth of 30 cm were taken the day before maize planting using a soil auger. Three separate cores were taken at random from each plot, bulked, and analyzed for NO_3–N using calcium phosphate extraction and for P using the Bray P1 method (Gelderman et al., 1987). Soil samples were also extracted with DTPA (Lindsay and Norvell, 1978) and analyzed for concentrations of K, S, Ca, Mg, Fe, Mn, and Zn using a inductively coupled plasma–atomic emission spectrometer (ICP–AES; Vista-MPX; Varian Instruments, Walnut Creek CA 94598).

Crop and Grain Measurements

Maize shoots were sampled for dry weight and mineral nutrient concentration when maize plants reached the 12 leaf (V12; Ritchie et al., 1992) development stage. Shoots were dried to constant weight at 60 C in a forced air oven, weighed, and ground in a Wiley mill (Thomas Scientific, Swedesburo NJ) equipped with a 1-mm screen. Ground tissue from each plot was combined and analyzed for N using the Kjeldahl method. Ground tissue was digested in nitric acid (Mars-X Extraction Unit, CEM Corp., Matthews NC) and an ICP–AES was used to measure P, K, S, Ca, Mg, Fe, Mn, and Zn concentrations.

A research plot combine (Massey Ferguson 8-XP; Kincaid Equipment Manufacturing, Haven, KS) equipped with an electronic weigh bucket was used to measure grain yield harvested from 4, 30 m long rows $plot^{-1}$. Harvested grain samples were measured for moisture and test weight using a grain analysis computer (Dickey-John GAC 2000, Johnston, IA). Grain yields were mathematically adjusted to 155 g kg^{-1} moisture content.

Kernel oil and starch were estimated with near infrared reflectance spectrometry (NIRSystems 6500, Foss Inc., Laural, MD). Grain was ground in a sample mill (Udy Cyclone, Seedburo Equipment Company, Chicago, IL) and kernel N was measured on ground tissue by dry combustion (CN 2000, LECO Corp., St Joseph, MI). Ground tissue was digested in concentrated nitric acid and analyzed for P K, S, Ca, Mg, Fe, Mn, and Zn concentrations using a ICP–AES (Vista-MPX; Varian Instruments, Walnut Creek CA).

Data Analysis

Soil, plant, and grain data sets were analyzed using PROC MIXED procedures appropriate for analysis for a split-plot experiment repeated over 2 yr (Littell et al., 2006). Year, N input, and rotation were considered as fixed effects while replication was considered a random effect. With the occurrence of a significant test for main effects, means were separated using the adjust = Tukey option in LSMEANS. Rotation × N input level interactions were further investigated by plotting the data means on bar graphs and calculating mean separations using the adjust = Tukey option in LSMEANS appropriate for testing three N input levels within each rotation level.

Canonical discriminant analysis, a dimension-reduction technique (Tabachnick and Fidel, 1996), was used to analyze combined nutrient soil, plant, and grain data. Given a classification variable with several groups as dependent variables (e.g., years, N input, or crop rotation treatments) and several quantitative independent variables (e.g., soil, plant, and grain nutrients), canonical discriminant analysis derives canonical discriminant functions which are linear combinations of the quantitative variables that have the largest possible multiple correlation with the groups in the classification variable. Used in this manner, canonical discriminant analysis is a powerful tool in determining the multivariate distances between these groups and their characteristics based on the independent variables (Duarte Silva and Stam, 1995).

Canonical discriminant analysis was used to determine (i) whether multivariate statistically significant differences exist between years, between N-inputs, and between crop rotations based on all nutrients in soil, plant, and grain, and (ii) which of the soil, plant, and grain nutrients account the most to these differences. The analysis was accomplished by first transforming the data to achieve a multivariate normal distribution and then by extracting one (in the case of years) or two (in the case of N input or crop rotation treatments) canonical discriminant functions from the 27 independent variables (i.e., nine nutrients measured each on soil, plant, and grain samples). A multivariate test statistic, Wilks' λ, equivalent to the univariate F statistic, was calculated for each nutrient, soil, plant, and grain source and for all three classification variables (i.e., years, N input treatments, and crop rotation), and tested for significance. Nutrients with significant ($P = 0.05$) Wilks' λ were identified and the corresponding R^2 values, which represent the amount of variation accounted for by that nutrient in discriminating between years, N inputs, or rotations, were listed. The larger the R^2 value, the greater the contribution of the respective variable to discrimination between groups.

The accuracy of each discriminant model was tested by developing "classification functions" (i.e., linear combinations of independent variables) for each sample to determine to which group (e.g., C-C, C-S or C-S-W/A-A crop rotation) it most likely belongs using those independent variables with significant Wilks λ values. A classification matrix was then developed for each discriminant model and the percent correct classification was calculated to verify the accuracy of the statistical probability of assigning each sample to the group it belongs to based on its respective discriminant model.

The R^2 values calculated above do not reveal the specifics of group discrimination by the respective canonical discriminant functions. To attain this information, the individual correlation coefficients between each independent variable and the first two canonical discriminant functions (i.e., CAN1 on the x axis and CAN2 on the y axis) were plotted and used to interpret the nature of discrimination between N input and between crop rotation treatments. The percentage of the total variation explained by each CAN was also listed on axis label of the graphs. The larger the value of the percentage of total variation explained, the greater the contribution of the respective canonical discriminant function to discrimination between groups.

Dotted lines were used to mark the origin of each of the two discriminant functions on the graphs. The correlation between the individual nutrient variables and the canonical function for each axis (i.e., loading) were also listed on the graph axis labels. These loading values, used in conjunction with treatment groups that are separated across the origin, allow the reader to determine the characteristics of the mineral nutrients in separating treatments. For example, for a particular nutrient that had negative loading on the discriminant function where treatments were separated across the origin on the graph, the reader can conclude that the negative-loading nutrient would be of lower concentration in treatments that discriminate on the positive side of the origin and will be of higher concentration in treatments that discriminate on the negative side of the origin. The opposite would be true for nutrients that have positive loading values. The Discriminant Analysis module in STATISTICA Release 8 (StatSoft, 2008) was used to carry out canonical discriminant statistical analysis and to construct 2-dimensional plots of the canonical discriminant functions.

RESULTS AND DISCUSSION

Growing Season Environment

The 1998 growing season had above normal air temperature in May and September, below normal in June, and near normal for July and August (Table 1). Rainfall was considerably below normal over the entire 1998 growing season with the exception of above normal rainfall recorded in August. Pan evaporation was greater in May through July than in August and September. In 1998, above average May air temperatures, timely rains, and low pan evaporation in August combined to produce maize yields across rotation and N input treatments that were 1.01 Mg ha^{-1} greater than the 12 yr average (Pikul et al., 2005).

During 1999, above normal air temperatures recorded from May through July were followed by near normal temperatures in August and September. Rainfall was below normal from June through August while above normal rainfall was recorded in April, May, and September. Pan evaporation was greater in June through August than in May or September. Warm air temperatures and above normal rain in April and May as well as near normal rainfall in July combined to produce maize yields across rotation and N input treatments that were 0.78 Mg ha^{-1} greater than the 12 yr average (Pikul et al., 2005).

Planting-Time Soil Mineral Concentrations

Analysis of data combined over the 2 yr of the study revealed that soils under the C-S-W/A-A rotation had significantly greater NO_3–N concentration and significantly less extractable P concentration compared with the soils under the other rotation treatments (Table 2). Observation of increased soil NO_3–N levels following alfalfa, likely due to the mineralization of N in legume organic matter residues (Power et al., 1986), confirm previous findings that net soil N mineralization following alfalfa was 30 to 40% greater than that following maize or soybean (Carpenter-Boggs et al., 2000). Extractable P concentrations previously have been shown to be lower in rotations that include multiple years of hay harvest compared with rotations where only grains were harvested (Karlen et al., 2006). Karlen et al. (2006) speculated that greater P removal with the forage crop compared with the grain crop was the cause of reduced soil extractable P concentrations following forages. In our study, P starter fertilizers were soil-applied to all of the grain crops used in the rotation treatments while the alfalfa phase of the C-S-W/A-A rotation received no fertilizer. We speculate that the observed reduction of extractable P in the C-S-W/A-A rotation was the result of greater P removal by the forage alfalfa crop and less P fertilizer applied to the soil.

Contrary to soil extractable P concentration, which was not affected by year, soil NO_3–N concentration was significantly ($P = 0.01$) greater in 1998 (12.1 mg kg^{-1}) than in 1999 (7.0 mg kg^{-1}). Because NO_3–N is mobile in the soil solution (Sugita and Nakane, 2007), the decreased concentration recorded in 1999 probably reflects increased NO_3–N leaching to below the 30 cm soil depth due to the greater rainfall received before planting in 1999 compared with 1998 (Table 1). Phosphorus, which is not as mobile in the soil solution as NO_3–N (Eghball and Sander, 1989), would be less susceptible to leaching during a wet year than NO_3–N.

Soils under high N input had significantly higher extractable P, K, Ca, and S concentrations compared with soils given no N inputs (Table 3). Values from the intermediate N input generally fell in between those of the high N and no N input treatments. Conversely, extractable Mn and Zn concentrations were less under intermediate N input compared with no N input while high N input values for these dependent variables tended to fall in between those of the no N and intermediate N treatments (Table 3). There were no significant effects of year on these dependent variables, with the exception of S (7.1 and 8.6 mg kg^{-1} in 1998 and 1999; $P = 0.001$) and Mn (31.6 and 24.6 mg kg^{-1} in 1998 and 1999; $P = 0.0006$). There were no significant rotation × N input level interactions for the soil mineral concentrations measured in this study.

Visual Description of Maize Growth

Maize plants grown under the N input and rotation treatments used in this study showed consistent visual differences across the 2 yr of the experiment. At the tassel development stage (VT; Ritchie et al., 1992), high N input treatments under all rotations produced excellent maize growth with no obvious mineral deficiency symptoms. Intermediate N input treatments produced good maize growth with no deficiency symptoms under the C-S and C-S-W/A-A rotations. The intermediate N/C-C treatment produced plants that had leaf chlorosis on older leaves. This chlorosis symptom, which started from the leaf tip and proceeded down the mid-vein, was consistent with N deficiency symptoms previously described for maize (Sprague, 1964). Plants were dark green with no chlorosis when grown under the no N input treatment under the C-S-W/A-A rotation, while those grown under no N input and C-S rotation had chlorotic older leaves indicative of N deficiency. Plants grown with no N input under C-C rotation were shorter, had older leaf chlorosis and necrosis, and showed a general leaf chlorosis on younger upper leaves. This combination of symptoms is consistent with previously-described severe N deficiency symptoms in maize.

During both years of the study, maize plants reached the VT development stage between 17 July and 26 July (DOY 198 to 207). Within this 9 d period, maize grown in C-C with no N input took longest to reach VT while maize grown in C-S-W/A-A rotation, regardless of N input treatment, reached VT soonest (data not shown). All plots during both years of this study were weed-free and maize plants showed no disease (rust) or insect (corn borer, corn rootworm) injury.

Table 1. Average monthly air temperature, total monthly precipitation, and pan evaporation for the 1998 and 1999 growing seasons near Brookings, SD.

Month	Year	Air temp.	Precip.	Pan evaporation
		°C	mm	
April	1998	7.2 (+0.5)†	46.2 (–6.4)	na‡
	1999	7.2 (+0.5)	106.2 (+53.6)	na
May	1998	16.7 (+3.5)	39.1 (–35.3)	203.5
	1999	14.4 (+1.2)	86.9 (+12.5)	160.5
June	1998	17.2 (–1.4)	51.8 (–58.4)	174.0
	1999	18.9 (+0.3)	65.5 (–44.7)	184.4
July	1998	21.1 (–0.4)	40.4 (–43.9)	208.5
	1999	22.8 (+1.3)	69.1 (–15.2)	199.1
August	1998	20.5 (+0.5)	89.2 (+17.8)	162.6
	1999	20.0 (0.0)	47.2 (–24.2)	185.2
September	1998	17.8 (+3.3)	19.3 (–47.7)	160.8
	1999	14.4 (–0.1)	72.1 (+5.1)	139.2

† Values in parentheses represent departure from normal (30-yr average).

‡ Data not available.

Table 2. Soil nitrate N and P concentrations under different crop rotation treatments as determined from 0- to 30-cm soil samples taken at planting time. Values represent data combined over N input treatments for both years of the study.

Rotation	NO_3–N	P
	mg kg^{-1}	
C-C†	7.9 b‡	10.6 a
C-S	8.5 b	10.5 a
C-S-W/A- A	12.3 a	8.6 b

† Rotation treatments: C-C is maize monoculture; C-S is maize-soybean 2-yr rotation; C-S-W/A-A is maize-soybean-spring wheat underseeded with alfalfa-alfalfa 4-yr rotation.

‡ Means followed by the same letter within columns are not significantly different (Tukey's means separation test, $P = 0.05$).

Maize Shoot Dry Weight Accumulation and Mineral Concentrations

Maize plants reached the V12 (Ritchie et al., 1992) developmental stage between 6 and 14 July (DOY 187–195) during both years of the study. When measured at the V12 stage, shoot dry weight was significantly affected by N input level (39.0, 43.7, and 42.7, g $shoot^{-1}$ for no N, intermediate N, and high N input treatments; $P = 0.0003$) and rotation (38.4, 41.6, and 45.2 g $shoot^{-1}$ for C-C, C-S, and C-S-W/A-A; $P = 0.01$) treatments. The lack of a significant N input × rotation interaction suggests that shoot dry weight under the different N input levels responded similarly across the three rotation treatments. A significant year effect for shoot dry weight (38.0 g $shoot^{-1}$ for 1998 and 45.5 g $shoot^{-1}$ for 1999; $P = 0.0003$) suggests that

Table 3. Soil mineral nutrient concentrations in different N input level treatments as determined from 0- to 30-cm soil samples taken at planting time. Values represent data combined over rotation treatments for both years of the study.

N input level	P	K	Ca	S	Mn	Zn
			mg kg^{-1}			
High	10.9 a†	145.3 a	3225.7 a	8.2 a	28.0 ab	0.49 a
Intermediate	9.4 b	129.0 b	3412.9 a	7.8 ab	25.0 b	0.36 b
None	9.4 b	129.9 b	2842.8 b	7.6 b	31.1 a	0.54 a

† Means followed by the same letter within columns are not significantly different (Tukey's means separation test, $P = 0.05$).

crop growth conditions up to the V12 crop development stage were slightly better in 1999 than in 1998.

Between the V6 and V12 leaf development stages, maize shoots rapidly increase in dry matter (Ritchie et al., 1992). Nutrient deficiencies at V12 leaf development stage may reduce grain yield by reducing the number of kernels ear^{-1} and the size of the ear at harvest (Ritchie et al., 1992). Shoot N concentration showed significant N input × rotation interaction ($P = 0.01$) suggesting that this dependent variable responded differently to N input treatments within the three rotations treatments studied. When compared with the high N input treatments, maize shoots grown with no N input had less N concentration under C-C and the C-S rotation treatments, while shoot N concentrations were not affected by N input level treatments under the C-S-W/A-A rotation (Fig. 1). These shoot N concentration values support the previously discussed visual observations of N deficiency symptoms of VT plants.

Shoot concentrations of several mineral elements also showed significant N input × rotation interaction (P, $P = 0.05$; K, $P = 0.009$; Ca, $P = 0.006$; Mg, $P = 0.05$; Zn, $P = 0.005$) suggesting that these dependent variables responded differently to N input treatments within the three rotations treatments studied. Significantly greater shoot K concentrations were measured in the no N input treatment compared with the intermediate N input treatment under C-C while no significant differences in shoot K concentration were present across N input level treatments in the C-S and C-S-W/A-A rotation treatments (Fig. 1). Shoot Ca and Mg concentrations were not significantly different across the N input level treatments for the C-S and C-S-W/A-A rotations but were significantly lower in the no N input treatment when compared to the intermediate N input level treatment under the C-C rotation treatment (Fig. 1). Shoot concentrations of P and Zn also were not significantly different across the N input treatments for the C-S and C-S-W/A-A rotations but were significantly higher in the no N input treatment compared with the high N input treatments under the C-C rotation treatment (Fig. 1).

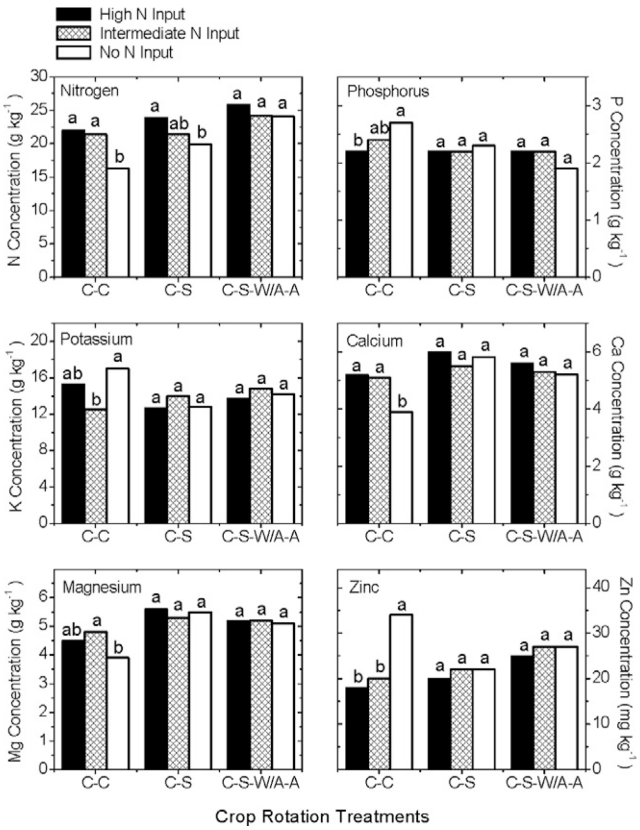

Fig. 1. Mineral concentrations at the V12 development stage for maize grown in different rotations and N input levels. Values represent data combined over the 2 yr of the study. C-C = continuous maize monoculture, C-S = maize soybean 2-yr rotation, C-S-W/A-A = maize soybean wheat/alfalfa alfalfa 4-yr rotation. Values within mineral elements and crop rotation treatments marked with the same letter are not significantly different (Tukey Test in Proc Mixed, α = 0.05).

Viewing the graphs for the significant N input × rotation interactions (Fig. 1) reveals trends and commonalities in how shoot mineral element concentrations reacted to the treatments. Concentrations of N, Ca, and Mg all appeared to be less at the no N input treatment for the C-C rotation compared with the other N input treatments for this rotation (Fig. 1). It is likely that decreased shoot N concentration in this treatment combination was the result of low soil NO_3–N concentrations for the C-C treatment (Table 1) and the fact that no fertilizer N was applied to these plots. Application of intermediate or high levels of N fertilizer resulted in significantly higher shoot N concentrations under the C-C treatment (Fig. 1) which in turn prevented the development of severe N deficiency symptoms previously described for the VT plants. The reasons for the Ca and Mg shoot concentration reductions (Fig. 1) are less evident. Because root Ca and Mg uptake occur in apical zones on the main and lateral axes (Ferguson and Clarkson, 1976), any process that interferes with the formation and growth of these root organs could also reduce the uptake of these divalent cations. Maize plants given no N fertilizer have been shown to have decreased root length while root mass is not affected (Anderson 1988), suggesting that the severe N deficiency in the no N input/C-C treatment combination may have resulted in decreased lateral root formation. Thus, we speculate that these reduced Ca and Mg shoot concentrations were the indirect result of reduced root growth in severely N-deficient maize.

Conversely, shoot concentrations of P, K, and Zn were greater in the C-C rotation for the no N input treatment compared with the other N input treatments (Fig. 1). Extractable soil concentrations of P and K were less under no N compared with the high N input treatment (Table 3) suggesting that enhanced shoot concentrations for P and K were not the result of changes in the soil concentration of these elements. However, P and K starter fertilizers were applied in a band near the seed furrow. Localizing P in a band, which causes increased root growth in the fertilized soil volume (Jackson et al., 1990), will enhance root P uptake as well as K uptake if K fertilizers are also present in the band (Barber, 1984). Decreased shoot dry weight accumulation due to poor N soil fertility, if P and K uptake were not greatly affected, would lead to increased shoot P and K concentrations (Jarrell and Beverly, 1981; Riedell et al., 1998). Increased shoot Zn concentration may simply reflect the higher soil extractable Zn concentrations (Table 3) found in the no N input treatment which, in turn, would increase Zn availability to plants (Catlett et al., 2002).

Kernel Composition, Grain Yield, and Mineral Nutrients

Kernel N, starch, and S concentrations were significantly affected by N input level (N, $P = 0.0001$; starch, $P = 0.0001$; S, $P = 0.0001$) and rotation (N, $P = 0.0001$; starch, $P = 0.002$; S, $P = 0.0008$) treatments. Kernel N and S increased while starch decreased with increasing N input treatments (Table 4). The C-S-W/A-A rotation produced grain that had significantly greater N and S concentrations but significantly less starch than the other rotation treatments (Table 5). Kernel oil concentration was also significantly ($P = 0.05$) higher in the C-S-W/A-A rotation treatment compared with the other rotation treatments (Table 5). Year had no significant effect on kernel N, starch, S, or oil concentrations and there were no significant N input × rotation interactions for kernel S, starch and oil concentration data. There is a close association between N and S concentration in maize (Kang and Osiname, 1976) where a ratio of 16:1 for N/S is considered to represent S sufficiency (Stewart and Porter, 1969). For maize kernels in this study, the N/S ratio ranged from about 14.5 to 16.

Maize kernels had greater oil concentration and less starch concentration under the C-S-W/A-A rotation compared with the others rotations (Table 5). These results support the findings of Alexander and Lambert (1968) who demonstrated that increased maize kernel oil concentration was accompanied by decreased kernel starch concentration. More than 90% of the kernel oil is located in the germ (Lambert et al., 1998) and oil concentration is positively correlated with an increased germ to endosperm ratio in the maize kernel (Brunson et al., 1948; Miller and Brimhall, 1951). Thus, our results may have occurred because the rotation treatments affected the germ to endosperm ratio of individual kernels. We speculate that the C-S-W/A-A rotation may have produced kernels with a larger germ to endosperm ratio than the other rotations studied. Additional data are needed to support this speculation.

Maize grain yield was significantly affected by N input ($P = 0.0001$) treatments. Grain yields in the intermediate and high N input treatments were significantly greater compared with the no N input treatment (Table 4). This plateau of grain yield in response to increased N input, coupled with the observation that kernel N concentration significantly increased as N input increased (Table 4), confirms the well-known principle that kernel N concentration increases rapidly in response to excess soil N supply after the yield response levels off (Deckard et al., 1984).

Crop rotation also significantly ($P = 0.01$) affected grain yield. Grain yields in the C-S and C-S-W/A-A rotation treatments were greater than the C-C rotation (Table 5). While there was no difference in the grain yield between the C-S and C-S-W/A-A rotation treatments, kernel N was significantly greater in the C-S-W/A-A than the C-S rotation treatment (Table 5). Increased kernel N concentration in the absence of increased grain yield suggests that the C-S-W/A-A rotation provided soil N in excess of that needed to increase yield (Deckard et al., 1984).

Significant N input × rotation interactions for grain yield ($P = 0.0001$) and kernel N concentration ($P = 0.0001$) suggest that these dependent variables responded differently to N input treatments under the different rotation treatments. Grain yield within the C-S-W/A-A rotation was not significantly different across N input levels while the no N input treatment resulted in lower grain yield within the C-C and C-S rotation treatments (Fig. 2). Also, kernel N concentration within the C-S-W/A-A rotation was not significantly different across N input levels while the no N input treatment resulted in lower N concentration compared with the other N input treatments within the C-C and C-S rotation treatments (Fig. 2).

Taken together, grain yield and kernel N concentration data suggest that N deficiency was reducing grain yields in maize grown under no N input treatments in the C-C and C-S rotation treatments while maize grown in the C-S-W/A-A rotation was N sufficient across all N input levels. We conclude that the residual soil N (Table 2) and mineralized N (Carpenter-Boggs et al., 2000) in the C-C and C-S rotation plots were insufficient to prevent N deficiency in the absence of fertilizer N. Application of N fertilizer alleviated this deficiency which resulted in increased grain yield and kernel N concentration in the C-C and C-S rotation treatments (Fig. 2). Thus, the importance of N fertilizer applications to monoculture maize or maize rotated with soybean is strongly demonstrated. Varvel (2000) and Pikul et al. (2005) also found that, in monoculture maize, a high rate of N fertilizer was required to achieve yields similar to those obtained in 4-yr rotational systems containing legume hay crops.

Significant N input × rotation interactions for kernel P ($P = 0.0003$) and K ($P = 0.0001$) were also present. Kernel P and K concentrations within the C-S and C-S-W/A-A rotations were not significantly different across N input levels while, in the C-C treatment, concentrations were greater in the no N input than in the intermediate N input treatment (Fig. 2). Because kernel P and K concentrations decreased with increasing N fertilizer input in the C-C treatment, we speculate that the severe N deficiency in the no N input level for the C-C rotation treatment reduced yield which in turn

Table 4. Grain yield and concentrations of N, starch, and S in grain harvested from plots managed under different N input treatments. Values represent data combined over crop rotation treatments for both years of the study.

N input level	Yield	N	Starch	S
	Mg ha^{-1}		g kg^{-1}	
High	6.7 a†	15.9 a	768.8 c	1.04 a
Intermediate	6.8 a	14.8 b	781.9 b	0.96 b
None	5.8 b	13.4 c	796.9 a	0.92 c

† Means followed by the same letter within columns are not significantly different (Tukey's means separation test, $P = 0.05$).

Table 5. Grain yield and concentrations of N, starch, oil and S in grain harvested from plots managed under different crop rotation treatments. Values represent data combined over N input treatments for both years of the study.

Rotation	Yield	N	Starch	Oil	S
	Mg ha^{-1}			g kg^{-1}	
C-C†	5.3 b‡	13.7 b	792.5 a	33.4 b	0.94 b
C-S	7.2 a	13.6 b	794.2 a	33.2 b	0.93 b
C-S-W/A- A	6.8 a	16.9 a	760.9 b	36.5 a	1.05 a

† Rotation treatments: C-C is maize monoculture; C-S is maize-soybean 2-yr rotation; C-S-W/A-A is maize-soybean-spring wheat underseeded with alfalfa-alfalfa 4-yr rotation.

‡ Means followed by the same letter within columns are not significantly different (Tukey's means separation test, $P = 0.05$).

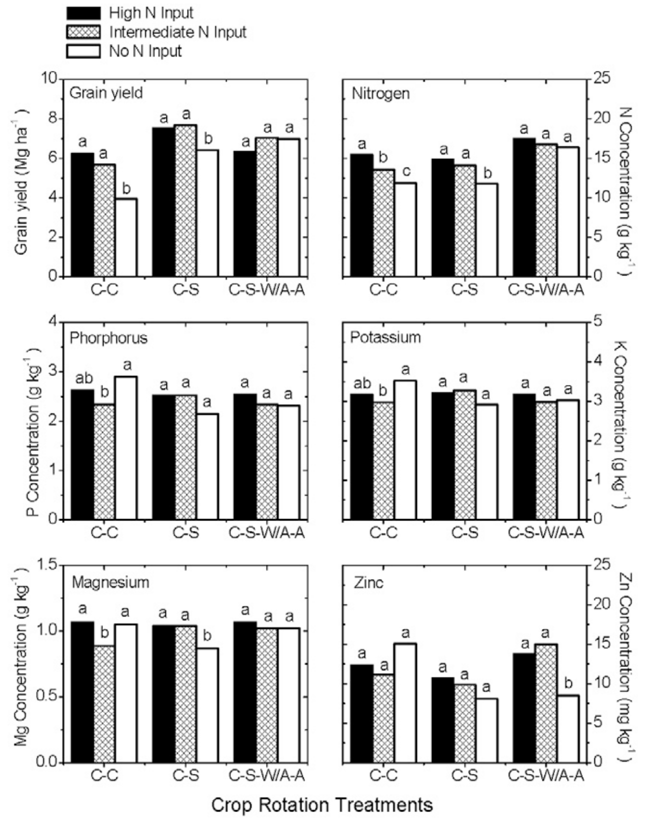

Fig. 2. Grain yield and kernel mineral concentrations for maize grown in different rotations and N input levels. Values represent data combined over the 2 yr of the study. C-C = continuous maize monoculture, C-S = maize soybean 2-yr rotation, C-S-W/A-A = maize soybean wheat/alfalfa alfalfa 4-yr rotation. Values within mineral elements and crop rotation treatments marked with the same letter are not significantly different (Tukey Test in Proc Mixed, α = 0.05).

prohibited the dilution of the P and K concentrations in the kernel (Jarrell and Beverly, 1981; Thiraporn et al., 1992).

Kernel Mg and Zn concentrations also were affected by significant N input × rotation interactions (Mg, $P = 0.02$; Zn, $P = 0.0001$). As with other kernel mineral elements studied, kernel Mg concentration was not affected by N input level under the C-S-W/A-A rotation (Fig. 2). In a manner similar to that seen for kernel N, kernel Mg concentration in the no N input treatment was significantly less compared with the other N input levels within the C-S rotation treatment. Under C-C, kernel Mg in the intermediate N input treatment was significantly less compared with the high N and no N input treatments (Fig. 2). Kernel Zn concentrations within the C-C and C-S rotation treatments were not affected by N input level while, in the C-S-W/A-A rotation treatment, Zn concentrations were significantly less in the no N input level compared with the intermediate N and high N input treatments (Fig. 2). Bruns and Ebelhar (2006) found that concentrations of Mg and Zn in maize kernels did not respond to N fertilizer treatments that increased overall grain yield. In contrast, Feil et al. (2005) found that kernel Mg concentrations were increased by N fertilizer treatments in 1 yr during a 3-yr study. These same authors suggested that a dilution effect caused by increased maize grain yield under increasing N fertilizer treatments resulted in reduced maize kernel Zn concentration. Thus, the data that we have presented as well as the contrasting observations reported in the literature do not explain the reasons for the complex interactions between N input level and rotation treatments for kernel Mg and Zn concentrations.

Multivariate Analysis of Mineral Nutrients in Soil, Crop, and Kernels

Canonical discriminant analysis is a statistical technique that allows the identification of variables that best discriminate between members of two or more groups (Duarte Silva and Stam, 1995). The R^2 values calculated for each mineral nutrient in soil, plant, and grain indicated the respective power of that nutrient in discriminating between years, between N inputs and between rotations (McGarigal et al., 2000; Table 6). The greater the R^2 value, the greater the discriminating power.

Different combinations of nutrients in soil, plant, and grain contributed to different levels of discrimination between years, between N inputs and between rotations. A total of 13 nutrients, albeit in different combinations, discriminated between years (100% correct classification) and between crop rotations (100% correct classification). Seven nutrients contributed to a lesser level of discrimination between N inputs (89% correct classification for no N input, 78% for intermediate N input, and 83% for high N input). Nutrients in grain (14 nutrients) were the most important in this discriminant analysis, followed by nutrients in soil (12 nutrients); whereas nutrients in shoots (seven nutrients) were the least important (Table 6).

Plant N was most discriminatory while soil N and soil P were least, out of 13 nutrients with significant R^2 values discriminating between years (Table 6). Out of seven nutrients with significant R^2 values for N inputs, grain N was most discriminatory while soil P was the least. If $R^2 = 0.50$ is considered as an arbitrary level of "important" power of discrimination, all 13 nutrients with significant R^2 for rotations contributed to the full discrimination between rotations.

The first and second canonical discriminant functions (CAN1 and CAN2) extracted from the whole data set for N input treatments accounted for 68 and 32% of total variation, respectively

Table 6. Significant R^2 values for mineral nutrients from soil, V12 maize shoots, and grain used to discriminate between years, between inputs, and between rotations for the 2-yr N input/crop rotation study. All mineral nutrient variables from the soil, plant, and grain data sets across years, N input, and rotation treatments were included in this analysis.

Nutrient	Years			N Inputs			Rotations		
	Soil	Plant	Grain	Soil	Plant	Grain	Soil	Plant	Grain
Nitrogen	0.36†	0.88		0.42		0.75			0.75
Phosphorus	0.38			0.29				0.56	0.97
Potassium			0.59			0.61			0.94
Calcium	0.68	0.77					0.57	0.80	
Magnesium							0.56		0.95
Iron			0.49				0.89		
Manganese			0.48				0.90		0.53
Sulfur	0.42	0.50	0.78						0.76
Zinc	0.58		0.49	0.35	0.47	0.52		0.76	

† With the occurrence of a significant Wilks' λ value ($P = 0.05$), the corresponding R^2 values were listed in the table.

(Fig. 3). The discriminant analysis indicated large negative loadings (i.e., correlation between the nutrient and a particular canonical function) of plant N, grain N, and grain S as well as large positive loading of plant Zn on CAN1. Negative loadings of soil Zn and soil P as well as positive loadings of soil Ca and soil N were evident on CAN2. The scatter plot of high N and no N input treatments were mostly separated along CAN1 (Fig. 3). Intermediate N input was separated from the high N and low N inputs along CAN2. This indicates a stronger separation between high N and no N input treatments, and a smaller separation between these two N input treatments and the intermediate N input treatment. The scatter plot and loadings on CAN1, in particular, indicate that large values of plant N, grain N, and grain S as well as small values of plant Zn were characteristics of high N inputs; whereas the opposite is true for low N inputs. Similarly, albeit with smaller discriminatory power on CAN2, large values of soil N and soil Ca were characteristic of intermediate N input; whereas, large values of soil P and soil Zn were characteristics of both high N and no N input treatments.

A stronger level of discrimination was observed between crop rotations when compared with the level of discrimination between N inputs. The C-C and C-S-W/A-A rotations, each of which were 100% correctly classified, were totally separated from each other along CAN1, which accounted for a large portion (85%) of total variation (Fig. 4). The C-S rotation, also 100% correctly classified, was scattered on both sides of CAN1 (Fig. 4). However, the C-S rotation was totally separated from the other rotations along CAN2, which accounted for 15% of total variation. Soil N, grain N, plant Ca, and grain S were most significant in differentiating between all three crop rotations on CAN1; whereas, grain N, grain P, grain S, grain Zn, and plant Ca were the most significant

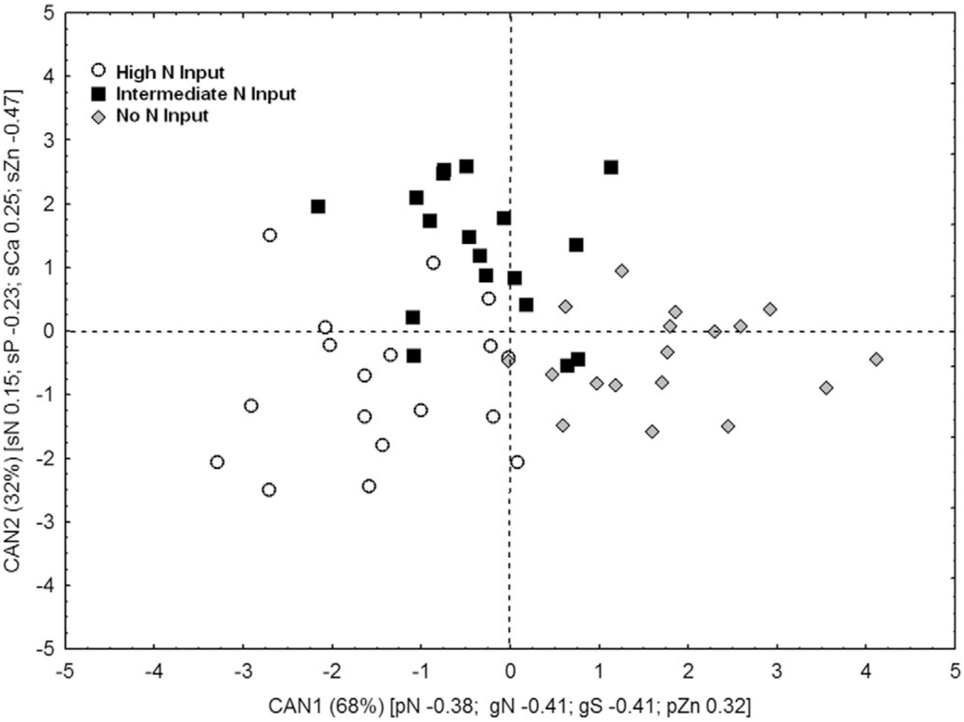

Fig. 3. Discriminant analysis plot of canonical discriminant functions derived from soil, V12 plant, and grain mineral nutrient data within N input treatments across years and rotation treatments. The dotted line marks the origin of each of the discriminant functions. Values in parentheses represent the total variation explained by each discriminant function. Loadings of mineral nutrients which contributed significantly to discrimination between N-inputs on each discriminant function are also presented on the *x* and *y* axis labels. (CAN1, first canonical discriminant function; CAN2, second canonical discriminant function; pN, plant N; gN, grain N; gS, grain S; pZn, plant Zn; sN, soil N; sP, soil P; sCa, soil Ca; sZn, soil Zn).

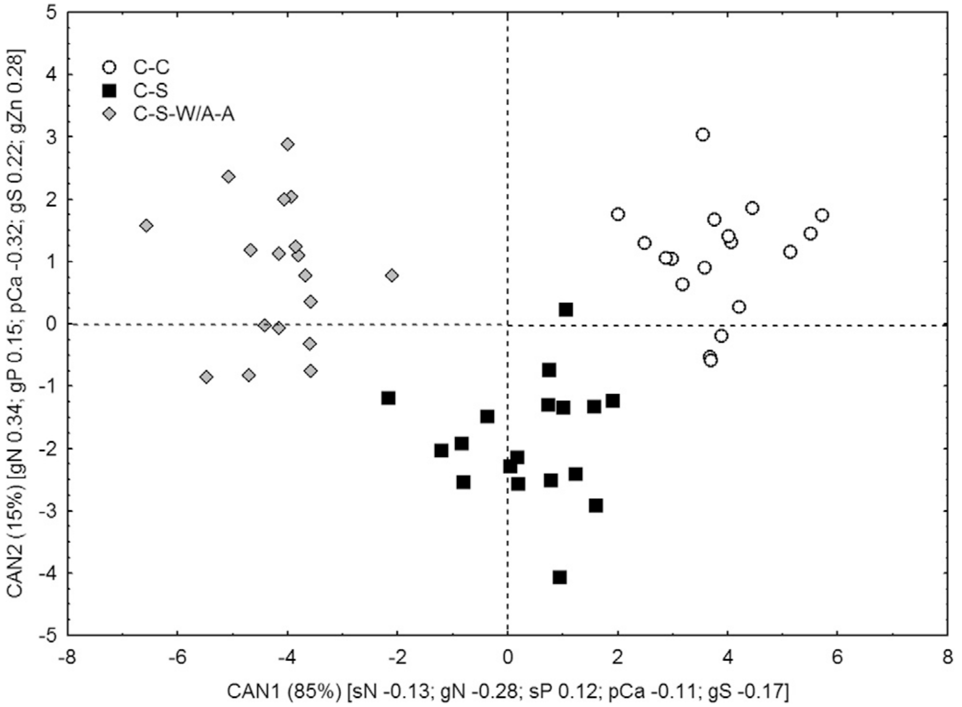

Fig. 4. Discriminant analysis plot of canonical discriminant functions derived from soil, V12 plant, and grain mineral nutrient data within rotation treatments across years and N input treatments. The dotted line marks the origin of each of the discriminant functions. Values in parentheses represent the total variation explained by each discriminant function. Loadings of mineral nutrients which contributed significantly to discrimination between crop rotations on each discriminant function are also presented on the *x* and *y* axis labels. (CAN1, first canonical discriminant function; CAN2, second canonical discriminant function; pN, plant N; gN, grain N; gS, grain S; pZn, plant Zn; sN, soil N; sP, soil P; sCa, soil Ca; sZn, soil Zn).

in differentiating between the C-S and the other two rotations. Small values of soil N, grain N, plant Ca, and grain S as well as large values of soil P were characteristics of the C-C crop rotation. Large values of soil N, grain N, plant Ca, and grain S as well as small values of soil P were characteristics of the C-S-W/A-A rotation.

Mineral Nutrient Relationships as Revealed by Univariate and Multivariate Analyses

Several common mineral nutrient responses to treatments were evident when the experiment was viewed at the univariate and multivariate levels. The importance of soil N likely resulted from the relationships between soil N fertilizer applications, the inclusion of legumes in rotations, and the effects legumes have on providing N-rich substrates for soil N mineralization. The importance of soil P likely resulted from the inclusion of a forage legume and the lack of P fertilizer applied to that phase of the C-S-W/A-A rotation which in turn altered the extractable levels of this important soil mineral element (Tables 2 and 3). Thus, it was not unusual to find that both soil N and soil P exhibited large loadings on canonical discriminant functions calculated for N input and crop rotation treatments (Fig. 3 and 4). Plant Ca responded to rotation treatment (and its interaction with N input) in a manner similar to that of plant N (Fig. 1). Additionally, plant Ca had large loading on both canonical discriminant functions (CAN1 and CAN2) important for discriminating between rotation treatments (Fig. 4). We speculate that N deficiency reduced root exploration needed to promote the absorption of Ca from the soil.

Given the effects of N input and rotation treatments (and their interactions) on grain N (Tables 4 and 5; Fig. 2), it was not surprising that grain N played an important role in discriminating between N inputs and between rotations. Grain N, as well as grain S, had large loading on CAN1 important for discriminating between N input treatments (Fig. 3) as well as on both CAN 1 and CAN2 important for discriminating between rotation treatments (Fig. 4). The similarity in loadings of grain N and grain S may have resulted because grain S responded to N input and rotation treatments in a manner similar to that of grain N (Tables 4 and 5). These data confirm a close association between N and S concentration in maize (Kang and Osiname, 1976).

Crop rotation has been an important component of agricultural systems for centuries (Crookston, 1984). With the advent of synthetic pesticides and fertilizers during the mid-20th century, however, extensive crop rotations were supplanted by intensive monoculture or short rotation cropping in many areas of the United States (Karlen et al., 1994). Concerns and costs associated with these intensely-managed systems include decreased soil organic matter, degraded soil structure, increased soil erosion, increased surface and groundwater contamination, and increased production costs (Bullock, 1992; Karlen et al., 1994). Our data suggest that under the 4-yr C-S-W/A-A rotation, where maize followed a forage legume, maize grain yield was stable across all N input levels studied. Conversely, maize yield decreased as N input level was reduced under the C-C monoculture and 2-yr C-S rotation treatments. Thus, growing maize in extended rotations that include forage legumes may be a more sustainable practice than growing maize in either monoculture or 2-yr rotation with soybean.

ACKNOWLEDGMENTS

The authors thank D. Schneider, J. Chlebecek, D. Harris, and M. Pravecek for excellent technical assistance and M. West, K. Rosentrater, and G. Varvel for critical review of an earlier version of this manuscript. Mention of commercial or proprietary products does not constitute endorsement by the USDA. The USDA offers its programs to all eligible persons regardless of race, color, sex, or national origin.

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